

## **Ultraviolet Absorbance at 254 nm for the Estimation of Organic Matter in Drinking Water (Based on Standard Method 5910B)**

### **Objective**

This protocol (standard operating procedure) is intended to provide guidance on the analysis of UV<sub>254</sub>-absorbing compounds. Additional information about analysis of UV<sub>254</sub> can be found in *Standard Methods for the Examination of Water and Wastewater* (1998) and in product information and operating procedures provided by the spectrophotometer manufacturer. Mention of commercial trade names are provided as examples and do not constitute an endorsement or recommendation from US EPA. Alison Gusses with EPA provided this protocol and subsequent example logsheet.

### **Operation of the Spectrophotometer**

Turn on the spectrophotometer (spec) approximately 30 minutes before analyzing samples to allow instrument to stabilize. Set the spec to the desired wavelength (254 nm). If a HACH (or similar) spec is being used, set to appropriate program number (e.g., HACH Program: 2640 Organics, UV-254).

### **UV<sub>254</sub> Sample Handling and Storage**

Samples (50 ml) should be collected into clean glassware and analyzed on the same day that they are collected. If same-day analysis is not possible, samples can be stored in the refrigerator for no more than 48 hours. Samples should be room temperature when analyzed; otherwise, condensation may form on the spec cell, possibly resulting in erroneous readings.

### **Sample Cell Selection**

Either a 1-cm square or a 5-cm cylindrical quartz cell should be used for measurement of UV<sub>254</sub> (1 and 5 cm describe the pathlengths, or distances, that the UV light travels through the cell). Cells should be selected such that the absorbance reading (filled with sample) is between 0.009 and 0.900. The measurement should be recorded as the absorbance value over 1 or 5 cm. This value should then be reported in units of  $\text{cm}^{-1}$  or  $\text{m}^{-1}$ . Regardless of which cell is used, the same cell size should be used for the entire sample set, (i.e., the absorbance readings remain between 0.009 and 0.900 for the entire sample set). Additionally, it is important that the cells are placed into the spec with the same orientation for every measurement (e.g., writing on cell

facing forward, left-facing, etc.), to minimize interference in the absorbance reading due to imperfections in the cells.

### Sample Filtration

1. All samples should be filtered prior to analyzing for UV<sub>254</sub> (e.g., using a vacuum, hand syringe or gravity filtration system).
2. A fresh filter should be used each day for UV<sub>254</sub> analysis, although the same filter can be used for multiple samples (as long as the samples are analyzed from “cleanest” to “dirtiest”). Depending on the number of samples being analyzed and the particulate content of the samples, multiple filters may be needed. A new filter should be used when the sample begins to filter slowly.
3. Set-up filtration system using clean glassware. If using a vacuum system, run 500 mL of deionized (DI, or any other “laboratory clean”) water through the vacuum filter apparatus before measuring samples. If using a hand syringe or other filtration system, run approximately 250 mL of DI water through the filter. Collect the last ~ 50 mL of filtrate, and measure the UV<sub>254</sub> to verify that the filter is not leaching UV according to the **Evaluation of Filter Leaching** procedure below.

### Evaluation of Filter Leaching

1. Rinse and fill the quartz cell with *unfiltered* DI water. Completely dry the outside of the cell and verify that the surfaces are dry and clean (i.e., lint and smudge free). Additionally, there should not be any bubbles in the light path. If any bubbles are observed, gently tap the cell on the lab bench to remove them.
2. Insert the cell into the spec. Zero the spec. Note the orientation of the cell (e.g., writing left facing).
3. Discard the water used to zero the spec, and rinse and fill with the filtrate generated during filter rinsing. Dry and clean the cell and place the cell into the spec in the same orientation as before. If the filter is not leaching UV<sub>254</sub> compounds, the absorbance reading should be between -0.009 and 0.009, and sample filtration can begin. If the reading is not in that range, this suggests that the filter is leaching UV<sub>254</sub> compounds and another 100 mL of DI water should be passed through the filter. Measure the UV<sub>254</sub> of the last 25-50 mL of filtrate. Repeat this procedure until it is confirmed that the filter is not leaching.

Once it has been verified that the filter is not leaching: Starting with the “cleanest” (i.e., with the lowest UV<sub>254</sub>, or TOC) sample, rinse the filtration glassware (e.g., syringe, filtration cup/funnel) with the sample. Next, filter a small volume (e.g., 25-100 mL) into the filter flask or collection beaker, rinse the flask or collection beaker with this filtrate and discard the filtrate. Filter the remaining sample for UV<sub>254</sub> analysis. The filtered sample should either be analyzed immediately from the flask, or poured into a clean beaker and set aside for UV<sub>254</sub> analyses. Rinse the flask or collection beaker with DI between sets of samples (between CFE, Settled, Raw). Repeat this procedure with the next-cleanest sample, and end with the “dirtiest” sample. If the filter becomes clogged (usually with a Raw sample) and water no longer passes through, begin with a new filter that has been properly rinsed with DI water.

### **UV Sample Analysis Procedure**

1. If the spec was zeroed during the **Evaluation of Filter Leaching** procedure, skip to step 2. Otherwise, rinse and fill the selected cell with *unfiltered* DI water. Completely dry the outside of the cell and verify that the surfaces are dry and clean (i.e., lint and smudge free) and that there are no bubbles in the light path. Insert the cell into the spec. Zero the spec.
2. Remove the cell and discard the sample used to zero the spec. Rinse and fill the cell with the first filtered (cleanest) sample. Dry and clean the cell and place the cell into the spec in the same orientation that was used to zero the cell. Record the absorbance once the spec yields a stable reading.
3. Repeat step 2 at least once with the same sample. The absorbance of the two measurements should be very similar (i.e., within ~ 0.003 nm). If not, repeat the measurement until this criteria is met. Report the UV<sub>254</sub> as the average of all readings.
4. Measure remaining samples according to steps 2 and 3. After every 10<sup>th</sup> the absorbance of unfiltered DI water should be measured. An absorbance significantly different than zero (i.e., by more than 0.009 cm<sup>-1</sup>) may indicate that the spectrophotometer has drifted or that the sample cell needs to be cleaned. If necessary, re-zero the spec (and note this on the log sheet).

## Filter Types

Filter selection should be a function of the intended analysis for the filtered samples (e.g., UV<sub>254</sub> only, UV<sub>254</sub> and DOC). Considerations for filter selection include whether the filter has a 45-μm pore size, whether the filter might leach UV<sub>254</sub> or DOC, and issues such as filter cost and availability. Examples of filters that have demonstrated minimal leaching of UV<sub>254</sub>-absorbing components during an in-house leaching study include the Millipore Durapore HVLP filter and the Gelman GN-6 filter. These filters were not evaluated for DOC leaching during this study. Karanfil et al (2003) reported several filters that showed minimal leaching of UV<sub>254</sub>-absorbing compounds and DOC, including several hydrophilic polyethersulfone filters made by Osmonics and Gelman and a hydrophilic polypropylene filter made by Gelman. Additional information on the evaluation of 17 different filters is provided in that same reference.

## References

- Karanfil, T., Erdogan, I., and M. A. Schlautman, 2003. "Selecting Filter Membranes for Measuring DOC and UV<sub>254</sub>", *Jour. AWWA*, 95:3:86.
- [Standard Methods for the Examination of Water and Wastewater](#), 1998 (20<sup>th</sup> ed.). APHA, AWWA, and WEF, Washington.

An example UV 254 Logsheet is available on the next page (page 5).

**Example UV<sub>254</sub> Logsheet**

Water: \_\_\_\_\_

Date of analyses: \_\_\_\_\_ Date sample collected: \_\_\_\_\_

Type of Filter Used: \_\_\_\_\_

- UV<sub>254</sub>, m<sup>-1</sup> = A \* 100 (for 1 cm cell) = A \* 20 (for 5 cm cell), where A = absorbance at 254 nm
- Measure "cleanest" sample first – "dirtiest" sample last.

<b>ZERO SPEC</b>			
<b>Verify filter is not leaching</b>		Cell pathlength:	
A:	1 <sup>st</sup> replicate 2 <sup>nd</sup> replicate 3 <sup>rd</sup> replicate (if needed)	UV <sub>254</sub> :	1 <sup>st</sup> replicate 2 <sup>nd</sup> replicate 3 <sup>rd</sup> replicate (if needed)
Sample:	1 <sup>st</sup> replicate 2 <sup>nd</sup> replicate 3 <sup>rd</sup> replicate (if needed)	UV <sub>254</sub> :	Cell pathlength:
A:	1 <sup>st</sup> replicate 2 <sup>nd</sup> replicate 3 <sup>rd</sup> replicate (if needed)	UV <sub>254</sub> :	Cell pathlength:
Sample:	1 <sup>st</sup> replicate 2 <sup>nd</sup> replicate 3 <sup>rd</sup> replicate (if needed)	UV <sub>254</sub> :	Cell pathlength:
A:	1 <sup>st</sup> replicate 2 <sup>nd</sup> replicate 3 <sup>rd</sup> replicate (if needed)	UV <sub>254</sub> :	Cell pathlength:
Sample:	1 <sup>st</sup> replicate 2 <sup>nd</sup> replicate 3 <sup>rd</sup> replicate (if needed)	UV <sub>254</sub> :	Cell pathlength:
A:	1 <sup>st</sup> replicate 2 <sup>nd</sup> replicate 3 <sup>rd</sup> replicate (if needed)	UV <sub>254</sub> :	Cell pathlength:
Sample:	1 <sup>st</sup> replicate 2 <sup>nd</sup> replicate 3 <sup>rd</sup> replicate (if needed)	UV <sub>254</sub> :	Cell pathlength:
A:	1 <sup>st</sup> replicate 2 <sup>nd</sup> replicate 3 <sup>rd</sup> replicate (if needed)	UV <sub>254</sub> :	Cell pathlength:
Sample:	1 <sup>st</sup> replicate 2 <sup>nd</sup> replicate 3 <sup>rd</sup> replicate (if needed)	UV <sub>254</sub> :	Cell pathlength:
A:	1 <sup>st</sup> replicate 2 <sup>nd</sup> replicate 3 <sup>rd</sup> replicate (if needed)	UV <sub>254</sub> :	Cell pathlength: